Chromosomal Aberration and Histopathological Effect of Metronidazole-Induced Toxicity in Male Rat

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Abstract

One of the most world anti parasitic and antibacterial compound used is Metronidazole (MTZ),all Drug toxicity must be acceptable to relief patients and should cause less harm to human than infection itself. MTZ is potentially genotoxic to humans due to the following facts: it is a proven toxogenic to human cells as well as wide range of side effects including chromosomal aberration and reproductive toxicity. The study is designed to assess the cytotoxic and reproductive toxicity effects of two dose of MTZ (250 mg/kg b.wt group and 500 mg/kg b.wt group) orally by gavage for 30 days and compared with control group (given normal saline) and then examine chromosomes from bone marrow cells and testicular gametogenic activity of adult male albino rats.

Evident toxicity of both treatments of MTZ groups on chromosomal aberration (fragment, deletion and ring chromosome),with increased number of polyploid cells which were significantly increased ($P< 0.05$) by dose and time compared with control group. MTZ significant decrease in sperm activity and in deformed form ($P< 0.05$) .as well as reduced testes weight, also MTZ stimulate injured in seminiferous tubules especialy germinal epithelium .

Keywords: *Metronidazole Genotoxic Chromosomal aberration, Seminal Vesicle, Sperm, Testis.*

Introduction

Metronidazole, a nitroimidazole the trade name is flagyl or Nidagyl, involved in first option in the relief the inflammatory disorders of the digestive and vaginal infections, it is therapy to treated microaerophiles like Helicobacter pylori, protozoa infection including amoebiasis and giardiasis in addition to methanogenicarchaea[(1,2)]. are activated via its reduction through reducing nitro group when low oxygen, forming fragmentation of imidazole and cytotoxicity is related with the amount of metronidazole uptake. the Reduction happen by two routes, The inactivation reduction of metronidazole to non-toxic amino stable derivative is oxygen insensitive [(3)] . during reduction activation the drug MTZ uptake electrons leading to form ring division and produced cytotoxic derivatives, this lead to the MTZ action as electron acceptor and prevent the force of proton motion lead to decreasing ATP formation, at the beginning the MTZ produced free radical (nitroso) and derivative of hydroxylamine the high affinity of oxygen for e^- than MTZ from generates oxygen radicals which stimulate DNA strand breaks 1[(4,5)]. This drug is highly metabolized in liver and excreted by kidney and less with feaces[2], After absorbed by intestine canal and increased its concentration in tissues, will be metabolized highly in liver via oxidation and glucuronide formed and this highly water solubility than the Glucuronic acid is original substance and excreted via kidney and little amount with feaces[(6)], significantly MTZ have capability in reduced the amount of colonic oxidative damage to proteins with no any effect on liver and on the glutathione levels along the bowel [(7)] . This drug advised by clinicians for long period about 4-8 weeks in special disease as Crohn's disease,Chagas disease, endocarditis, osteomyelitis infection of deep neck, joint and liver abscess caused health problems on male fertility when studied on laboratory rats[(8)].

Objective(s): Aim of our study was to investigate the influenced of MTZ on cytogenetic and spermatogenesis

by observing the chromosomal aberration and histological changes of testes in adult male rats.

Material and Method

The drug we used ismetronidazole (flagyl) tablets 500 mg is produced by Novartis Pharma company Switzerland.

Experimental Animals: The types of rats in this experiments are 15 adult male (*Rattusnorvegicus*) weighting 200-250 gm in Faculty of Science, University of Kufa, in order to adapted rats to lab conditions we kept them in animal house at 24±28°C under 12:12 hr light and dark cycles (standard environment) for pellets food and water in the animal house. After 2 weeks of adaptation three groups, each one consist 5 rats, Group 1 depend on normal saline, group 2 received metronidazole 225mg/kg p.o. whereas groups 3, received 500mg/kg. offlagyl, prepared as homogenized suspension in normal saline, Animals were treated for 30-days

Bone marrow chromosome assay: After thirty days all animal injected with intrapretonealcolchicines (4mg/kg), to stop the division of cell . After two hours from givencolchicines, animals were sacrificed and preparation of chromosomes from bone marrow according to Alder 1984 [9,10], which includes aspirated the bone marrow from femur bone with one milliter of 0.075M KCl. The harvest cell preserved at 37°C for 20 minutes and centrifuged at 1000 rpm (10 min.) . Then cells fixed in Carnoys fixative e (methanol: acetic acid = 3:1) ratio and dropped from distance about 25 cm on cool clean slides to facilate the bursting of the cells. Then drying slides and stained with giemsa 10%,6.8 pH for 5 min. examined any abnormalities of chromosomes at magnification of 1000 X (100 X 10) .

Sperm abnormality assay: After animals sacrificed the epididymis excised,the sperms suspension was formed in 1ml saline. Then dropping suspension on clean slides and dried, fixed with absolute methanol, finally stained with hematoxylin (15 min.), the slides washed by the tap water, then using 1% Eosin for (5 min), after that slide washed by distilled water and drying slide [11].

Ethics: The collection of samples carry out within a specific barrier system in necropsy room in the animal house in science college of kufa university to minimize the suffering of animals.

Statics: Statistical analysis was done by using mega stat soft ware,Values are analysed at $p<0.05$.

Results

The data obtained from bone marrow examination at metaphase stage are in table 1. the significant differences among groups. By presence of chromosomal aberration as control group. The deletion aberration was highest value in Flagyl dose 500mg/kg and Flagyl 250mg/kg were 11.4±2.07 and 6.6±1.34 respectively, then fragmented chromosome 7.2±0.84 and 4.0±0.71 respectively while ring aberrations record low value in treated groups.

Table 1: Chromosomal aberrations of bone marrow cells of experimental rats (mean±S.D).

Group	Chromatid Break	Fragmented Chromosome	Aneuploidy	Deletion	Dicentric Chromosome	Ring
Control	0.0±0.0	0.2±0.45	0.0±0.0	3.4±1.34	0.0±0.0	0.0±0.0
Flagyl 250mg/kg	1.8±1.10	4.0±0.71	0.0±0.0	6.6±1.34	0.4±0.84	0.2±0.45
Flagyl 500mg/kg	3.8±1.92	7.2±0.84	2.6±0.89	11.4±2.07	1±1.73	0.8±0.4

The figure 1(A-E) shows many types of structural chromosomal aberrations, the line pointed translocation, breaks, chromatid gap, chromosome gap, acentric, ring, aneuploidy with thickening chromosomes, chromosomal association, chromatid gap and polyploidy respectively. The numerical aberrations appeared as polyploidy cells and an aneuploidy.

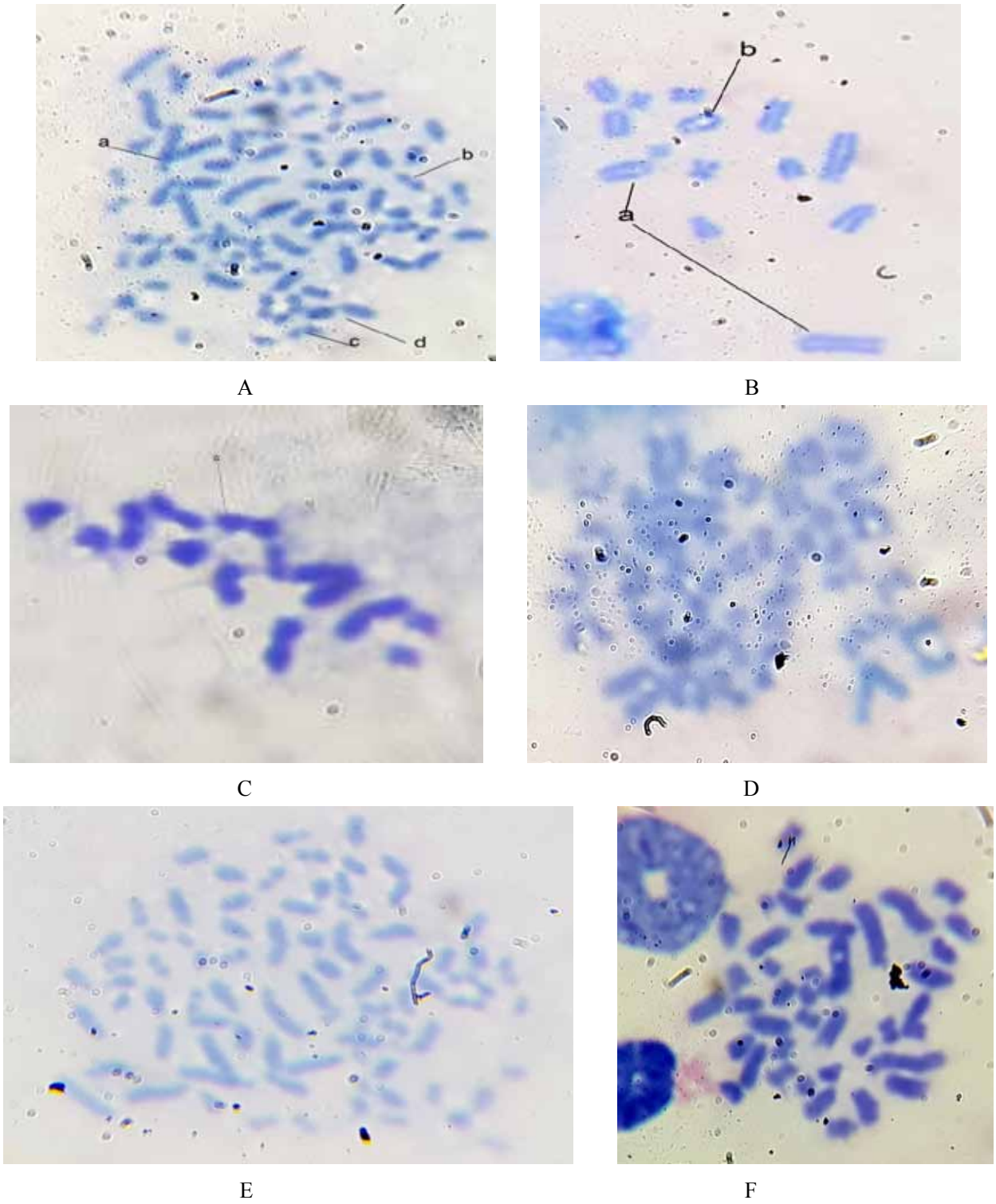
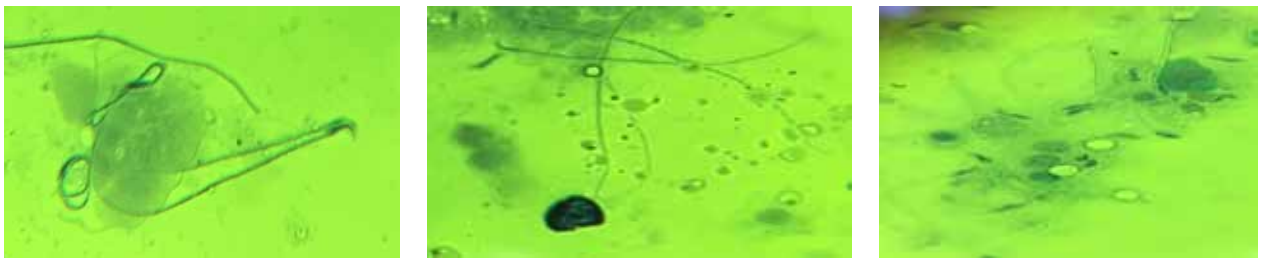


Figure (1): Metaphase stage of bone marrow treated rats with MTZ. A (a translocation b; chromatid break; c chromatid gap d; chromosome gap); B (a acentric b ring); C(aneuploidy with thickening chromosomes); D chromosomal association and chromatid gap): E polyploidy(chromosomal break); F (control group).

Sperm abnormality Assay: The results of the sperm abnormality are shown in the table 2. Show the most prominent deformations were folded or coiled filament, amorphous, flagellum with ansa, double head, double tail, coil with microcephaly, bent at cephalocaudal region, hookless flagella and multiple abnormalities as included in the Figure 2. The treatment with MTZ induced significant abnormalities than control ($P<0.05$).

Table 2: Changes in some sperm abnormality of experimental rats (mean±S.D).

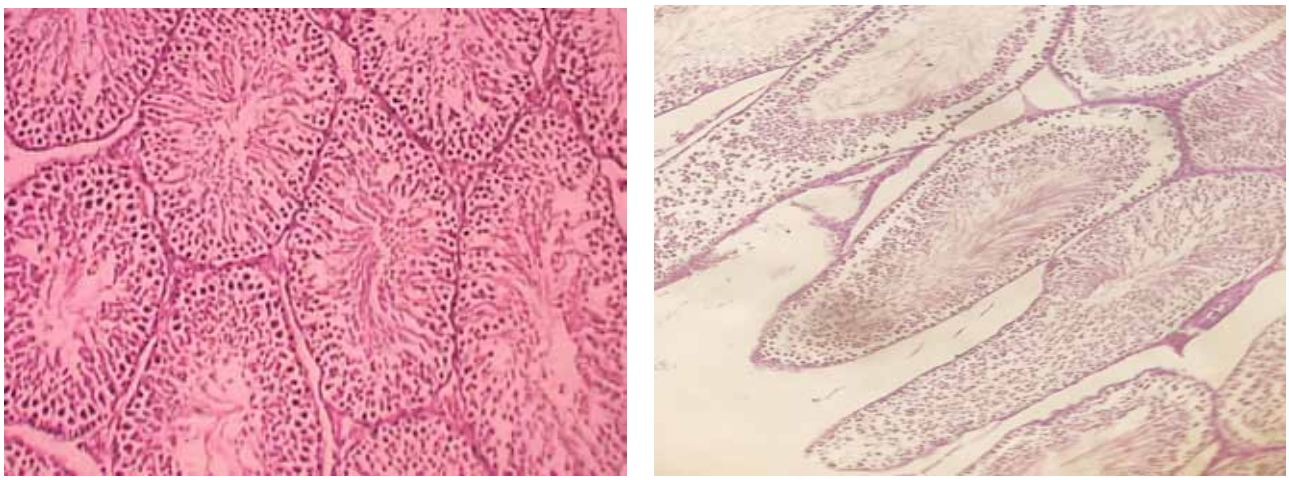
Sperm Abnormalities	Control	MTZ-250 mg/kg	MTZ-500 mg/kg	p
Normal	93.40+5.2	77.00+3.39	43+2.45	p<0.05
coiled/folded	5|+0.7	6.2+1.30	14.20+3.1	<0.05
Bent at cephalocaudal	0	7.6+1.67	9.3+2.12	<0.05
Flagellum with ansa	1+0.7	5.8+1.3	7.8+1.3	<0.05
Amorphous	0.8+0.84	3.4+1.02	9.40+1.14	<0.05
Multiple abnormality	0	9.40+2.3	11.20+1.3	<0.05
Double tailed	0	5+1.4	8.4+0.89	<0.05
Coiled with	0	8.60+0.55	14.80+1.10	
Hookless flagellum	0	4.40+0.55	8.20+0.84	



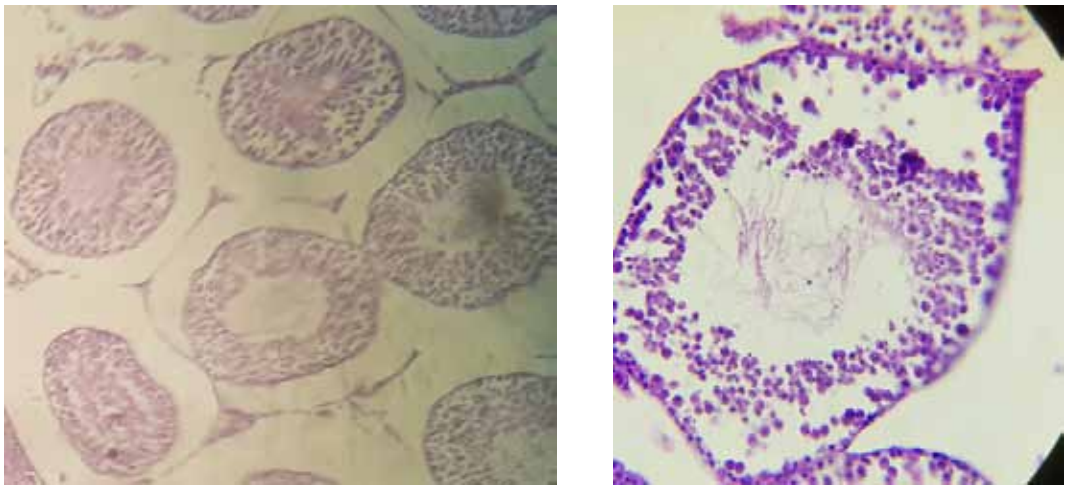
A B C

Figure (2): Deform shapes of sperms in treated rat with MTZ. A flagellum with ansa and folding; B abnormal sperm; C only head.

Histological Examination: Testes with seminiferous tubules of the control rats had normal appearance (A). but (B-D) of treated rats with MTZ (250, 500 mg/kg/day); (B) reveals the little effect of shrinkage at (250 mg/kg/day)dose,C-D MTZ500 mg/kg/day, show sloughing,depletion, vacuolization and disorganization of the cells like multinucleated giant cells in the seminiferous tubules.



A B



C D

Figure (3): Show section in testis of control (A) was normal of seminiferous tubules. (B-D) MTZ (250, 500 mg/kg/day)-treated rats: (B) shows the little change of the seminiferous tubules and atrophy of leydic cell(250 mg/kg/day),C-D MTZ500 mg/kg/day, sloughing,depletion, vacuolization and disorganization of the cellsatrophy and little Leydig cells .

Discussion

Genotoxicity tests able to detect drugs that cause genetic damage by interaction with other cellular targets, such as enzymes and microtubules, are particularly interesting This study assessed with toxicosis effect of MTZ on male rats based on chromosomal aberration, sperm abnormalities andhistopathologic alterations in testes tissue .depending on WHOInternational Agency for Research on Cancerthe MTZ considered as a possible carcinogen.[12] there was study denoted to chromosomal abnormalities in circulating lymphocytes in people with Crohn's disease treated with metronidazole.[13]

Chromosomal abnormalities are usually diagnosed structural changes and rearrangement *in vivo* at metaphase[14].

The mechanism explain the stimulation of chromosomes aberration include free radical production by two way first was auto-oxidationand second by enzyme-catalyzed oxidation of organic compounds, thiscaused peroxidation of lipidsof membranes in tissues then induced damage in DNA bases via formation covalent binding between the product of lipid peroxidation and DNA [15,16].

Our results show those genotoxic of receiving high dose of MTZ for long period about 30 days in expermental group male rats were significant increasing of chromosomal abnormalities in treated rats as comparing to control group in agreement with previous study (17),this drug depending on WHOInternational Agency for Research on Cancerthe MTZ considered as a possible carcinogen.[18]it s caused most chromosomal aberration like chromatid breaks due to change in chromosomal protein charge or DNA cross linking [19, 20]. This increased aberrant metaphase percent (21), A chromosome dicentric is one an aberrant chromosome having two centromeres, resulted in unusual behavior, a dicentric chromosome appear abnormal chromosome behavior during cells division lead to abnormal separation of chromosomes to daughter cells,both small breaks and a centric fragments (lacks centromere) couldn't survive to the next generation and the consequence of this lead to loss genetic material in these cells 22. The ring shape of chromosome result from part of chromosome has broken off and then sticky end fused to form ring, Interchanged between chromosomal segments, These aberration lead to loss or mistakes in genetic material, All these abnormality belong to attack DNA by the free radicals, besides some spots in purine, lead to base substitution and breakage of DNA, or mutation [23].

In present,MTZ the abnormal sperm significantly increased, the C3-chloro side – chain of the nitroimidazole ring(24,25) is one of the metabolites of ornidazole can

formed 3-chloro-lactaldehydethe and α-chloro-hydrin, are prevent work of the glycolytic enzymes like glyceraldehydes-3- Phosphate dehydrogenase (GAPDH) and triosephospahteisomerase (TPI) in the spermatozoa [(26)].this reduce ability of spermatozoa to get ATP by the glycolytic pathway [(27)].and then Spermatogenic cells would be injured by the increased inhibition of α– glycosidase malondialdeyde (MDA), while the less activity of sperm belong to energetic transferase diminished [(28)].

Our observed agree with[29] whom revealed in their experiments, after 700 mg/kg b.wt. as single dose of 2 thiazolyl-5- nitroimidazole caused infertility after 3 weeks in mice, then after 48 days return fertility maybe due to decreasing in circulating hormones LH,FSH and biosynthesis testosterone may affect the spermatogensis lead to diminished in activity motion of sperms and highly abnormalities sperm shape [(30)] .also there is signifigant differences in histological changing for testes of treated groups 250mg/kg and 500mg/kg than control group as figure 3,we found the little shrinkage of the seminiferous tubules(250 mg/kg/day),than MTZ500 mg/kg/day, as well as vacuolization, disorganization, depletion and sloughing of the germ cells and at high dose we found the evacuation of seminiferous convoluted tubules from sperms and limited number of Leydig cells at interstitial tissue between the seminiferous convoluted tubules,our study agree with [(31)],the reason belongs to formation of free radical this increased direct and indirect oxidative stress leading to cells destruction and this appearance resulted in LH and FSH depletion which in turn on spermatogensis[(30)]

Conclusion

From this study we have come up with a serious toxicity MTZ for high dose and long term on chromosomes structure DNA,histopathological effect on testis tissues and sperms shape in addition to motion sperms defect.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Biology and all experiments were carried out in accordance with approved guidelines.

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